Cytospora Canker on Colorado Peaches: Current Research

Jane E. Stewart
Plant Pathologist
Colorado State University
Bioagricultural Sciences and Pest Management
Cytospora canker

- Caused by fungal species *Cytospora*
- Opportunist pathogens, cannot invade healthy intact bark
Disease cycle

Fungus persists and forms fruiting structures in dead wood and cankers

Conidia spread by rain; germinate and infect injured and dead tissues, expands as cankers

Humid, wet

Leaf scars, damaged buds

Crotches with narrow branch angles

Unhealed pruning stub

Shaded winter damaged twig

Poorly healed wound

Canker rings formed by infection and host callus formation

Biggs and Grove 2006. Plant Health Instructor
Cytospora presence in Colorado

Surveyed
- 200 acres
- 42 varieties
- 2-32 year old orchards (mean 11 years)

Results
- 100% of orchards surveyed infected (mean 75% infected)
- Ave number of infections per tree was 5.2 (range 0-27)
- Currently analyzing relationships between practices and infection
Questions

• How many *Cytospora* species cause infections on peaches?
• What chemicals are effective against *Cytospora*?
  – Preventative vs. suppressive?
• Are trees susceptible year around?
Species differ biologically

• Three species have been identified on peach in other peach growing regions:
  – *Cytospora leucostoma*
  – *Cytospora paraleucostoma*
  – *Cytospora cincta*

• Variation in occurrence and virulence on different peach varieties

• Found on different locations of the tree
What species are found at Orchard Mesa and Rogers Mesa?

• Collections made in July-August 2015
  – 135 isolates were recovered at different elevations
  – Identified species based on DNA sequencing
One species found on Colorado peach

- *Cytospora leucostoma*

- No difference between orchards low vs. high elevation

- No difference between winter/summer isolates

- Apple and peach isolates genetically distinct
Risks of other species introduced into Colorado

• Virulence
  – Difference among *C. leucostoma*, *C. paraleucostoma*, *C. cincta*

• Growth at different temperatures

• Fungicide sensitivity

• Sporulation

• Differences with peach varieties
Questions

• How many *Cytospora* species cause cankers and gummosis?

• What chemicals are effective against *Cytospora*?
  – Preventative vs. suppressive?

• When are trees susceptible?
What chemicals are effective against Cytospora?

- Evaluate the efficacy of conventional and organic fungicides for *Cytospora leucostoma* control
- Test wound sealing alternatives to develop preventive and spore suppressive approaches in existing orchards
Chemical Testing Phases

**Laboratory Assay:**
- Testing chemicals *in vitro* on plates
- Testing chemicals on detached branches

**Field Trials:**
- Testing chemical sprays as preventive measure on branches
- Testing chemicals embedded in paints as preventive measure on branches
- Testing chemicals on existing cankers for spore suppression
## Conventional Chemicals Evaluated

<table>
<thead>
<tr>
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<th>Active ingredient</th>
<th>Label rate (per 200 gal.)</th>
<th>Rate chosen</th>
<th>Mode of Action</th>
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</thead>
<tbody>
<tr>
<td>Microthiol Disperss</td>
<td>Sulfur</td>
<td>10-20 lb</td>
<td>15 lb</td>
<td>Multi-site</td>
</tr>
<tr>
<td>Fontelis</td>
<td>Penthiopyrad</td>
<td>14-20 oz</td>
<td>17 oz</td>
<td>Respiration</td>
</tr>
<tr>
<td>Torino</td>
<td>Cyflufenamid</td>
<td>3.4 oz</td>
<td>3.4 oz</td>
<td>Unknown</td>
</tr>
<tr>
<td>Pristine</td>
<td>Pyraclostrobin &amp; Boscalid</td>
<td>10.5-14.5 oz</td>
<td>12 oz</td>
<td>Respiration</td>
</tr>
<tr>
<td>Aliette WDG</td>
<td>Fosetyl</td>
<td>10 lb</td>
<td>10 lb</td>
<td>Unknown</td>
</tr>
<tr>
<td>Topsin M WSB</td>
<td>Thiopthanate-methyl</td>
<td>1-1.5 lb</td>
<td>1.25 lb</td>
<td>Cytoskeleton/motor proteins</td>
</tr>
<tr>
<td>Benlate WP</td>
<td>Benomyl</td>
<td>24-32 oz</td>
<td>28 oz</td>
<td>Cytoskeleton/motor proteins</td>
</tr>
<tr>
<td>Captan</td>
<td>N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide</td>
<td>3-4 qt</td>
<td>3.5 qt</td>
<td>Multi-site</td>
</tr>
<tr>
<td>Inspire Super</td>
<td>Difencoconazole &amp; Cyprodinil</td>
<td>16-20 oz</td>
<td>18 oz</td>
<td>Protein synthesis</td>
</tr>
<tr>
<td>Ziram</td>
<td>Zinc dimethyldithiocarbamate</td>
<td>3 - 5.3 lb</td>
<td>1.15 lb</td>
<td>Multi-site</td>
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</table>
## Organic Chemicals Evaluated

<table>
<thead>
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<th>Treatment name</th>
<th>Active ingredient</th>
<th>Label rate (per 200 gal.)</th>
<th>Rate chosen</th>
<th>Mode of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl</td>
<td>CaCl</td>
<td>48 oz</td>
<td>48 oz</td>
<td>Multi-site</td>
</tr>
<tr>
<td>Neem Oil</td>
<td>Neem Oil</td>
<td>3 qt</td>
<td>3 qt</td>
<td>Not classified</td>
</tr>
<tr>
<td>Mpede</td>
<td>Potassium salts</td>
<td>2-4 gal</td>
<td>3 gal.</td>
<td>Multi-site</td>
</tr>
<tr>
<td>Kaligreen</td>
<td>Potassium bicarbonate</td>
<td>2.5-3 lb</td>
<td>2.75 lb</td>
<td>Not classified</td>
</tr>
<tr>
<td>Serenade</td>
<td>Bacillus subtilis</td>
<td>14-20 oz</td>
<td>17 oz</td>
<td>Lipid synthesis/transport</td>
</tr>
<tr>
<td>NuCop WP</td>
<td>Copper Hydroxide</td>
<td>8-20 lb</td>
<td>10 lb</td>
<td>Multi-site</td>
</tr>
<tr>
<td>Badge X2</td>
<td>Copper Hydroxide &amp; Copper Oxychloride</td>
<td>3.5-5.25 lb</td>
<td>4.25 lb</td>
<td>Multi-site</td>
</tr>
<tr>
<td>ZnSO4</td>
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<td>4-6 lb</td>
<td>5 lb</td>
<td>Multi-site</td>
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<td>Lime sulfur</td>
<td>Calcium polysulfide</td>
<td>20-24 gal.</td>
<td>22 gal.</td>
<td>Multi-site</td>
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</table>
Methods

*In vitro* chemical plates

1. Chemical treatments amended in agar solution, at commercial mid-rate

2. Isolates inoculated onto plates

3. Inoculated plates incubated at 25°C for 7 days

4. Colony areas assessed every 24 hours

*C. leucostoma* growth in chemically amended plates
Several Effective Conventional + Organic Chemicals

*In vitro* chemical plates

**Chemical Treatments**
- Conventional
- Organic
- Positive Control

<table>
<thead>
<tr>
<th>Chemical Treatments</th>
<th>Colony Area (mm²)</th>
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<tbody>
<tr>
<td>Microthiol D.</td>
<td>3500</td>
</tr>
<tr>
<td>Fontellis</td>
<td>2000</td>
</tr>
<tr>
<td>Torino</td>
<td>2500</td>
</tr>
<tr>
<td>Pristine</td>
<td>2000</td>
</tr>
<tr>
<td>Aliette</td>
<td>1500</td>
</tr>
<tr>
<td>Topsin</td>
<td>1500</td>
</tr>
<tr>
<td>Benlate</td>
<td>1500</td>
</tr>
<tr>
<td>Captain</td>
<td>1500</td>
</tr>
<tr>
<td>Inspire Super</td>
<td>1500</td>
</tr>
<tr>
<td>CaCl</td>
<td>3000</td>
</tr>
<tr>
<td>Neem Oil</td>
<td>5000</td>
</tr>
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<td>Positive Control</td>
<td>5000</td>
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(α = 0.05) Tukey’s HSD adjusted p-values: \( P < 0.05 \)
Methods
Detached branches, chemically submerged

1. Detached branches wounded then submerged in mid-rate chemical solutions for 5 minutes
   **Conventional chemicals:**
   - Aliette, Topsin, Benlate, Captan, Inspire, Ziram
   **Organic chemicals:**
   - Neem oil, Mpede, Kaligreen, Serenade, NuCop, Badge, ZnSO4, lime sulfur

2. *Cytospora* inoculated onto wounded branches

3. Lesion lengths assessed 8 days post inoculation
C. leucostoma lesion

C. leucostoma lesion
Several Conventional + Organic Chemicals Effective

Lesion Length (mm)

(α = 0.05)
Tukey’s HSD adjusted p-values: P < 0.05
Methods

Chemical field sprays

1. Wounds made on 1-year wood
2. Label mid-rate chemical sprays applied after wounding
   • Conventional: Topsin and Captan
   • Organic: Lime Sulfur and NuCop
3. Inoculation (Summer, Fall, Spring)
4. Branches harvested
Prune wound on 1 year old peach shoot

Chemical applications

Inoculation on peach shoot pruning cut
Opening *Cytospora* infected branch

*Cytospora* lesion in prune wound
Topsin and Captan Seasonal Efficacy

Months Post Inoculation (MPI)
- Conventional
- Organic
- Effective

(α = 0.05) (Tukey’s HSD adjusted p-values: P < 0.05)
NuCop Potential Phytotoxicity to Peach

Months Post Inoculation (MPI)
- Conventional
- Organic
- Effective

(α = 0.05) (Tukey’s HSD adjusted p-values: P < 0.05)
Methods
Chemical paint field sprays

1. Wounds made on 1-year wood

2. Label mid-rate chemical sprays applied after wounding
   - Conventional: Latex, Topsin and Captan
   - Organic: kaolin clay (Surround), Lime Sulfur and NuCop

3. Inoculation (Summer, Fall, Spring)

4. Branches harvested
Latex Combinations and Lime Sulfur Show Evidence of Seasonal Efficacy

Months Post Inoculation (MPI)
- Conventional
- Organic
- Effective

(α = 0.05) (Tukey’s HSD adjusted p-values: P < 0.05)
Most Effective Preventive Treatment?

- Conventional treatments:
  - Topsin
  - Captan
  - 50% Latex (Combinations)

- Organic treatments:
  - Lime Sulfur

Infected Prune Wound on Peach Tree
Chemical Testing Phases

Laboratory Assay:
Testing chemicals *in vitro*

Laboratory Assay:
Testing chemicals on detached branches

Field Trials:
• Testing chemical sprays as preventive measure on branches
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• Testing chemicals on existing cankers for spore suppression
Methods

Painting existing cankers

1. *Cytospora* cankers selected randomly in field

2. 10 ml of water (spore effluent) collected from cankers for pre-treatment measurements

3. Chemicals paint combinations applied to cankers:
   - **Conventional Chemicals:** Topsin + latex, Captan + latex, and latex
   - **Organic Chemicals:** Lime sulfur + Surround, NuCop + Surround and Surround

4. Spore effluent collected from cankers after chemical applications once a month for 7 months
Pre-treatment *Cytospora* canker

Latex treated *Cytospora* canker
Continuous Spore Production in all Treatments

- Significant spore decrease compared to pre-treatment (red) within treatment group

(\(\alpha = 0.05\)) (CI = 95%) (Tukey adjustment)
Questions

• How many *Cytospora* species cause cankers and gummosis?
• What chemicals are effective against *Cytospora*?
  – Preventative vs. suppressive?
• When are trees susceptible?
Cytospora can infect peach at any season.
Cytospora research summary 2016-2017

- *Cytospora leucostoma* was identified as fungal pathogen
- Chemical options for preventive and suppressive control
- Trees are susceptible year round
- The development of a molecular tool to study epidemiology
- Spores are dissemination through the year, when conditions are favorable
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* Rate was 3% Lime sulfur
Funding: USDA-CDA Specialty Crop  
February 2018 – November 2019

**Project title:** Cytospora management in peach orchards through cultural practices, cultivar selection, and stress mitigation

- **PIs:** Jane E. Stewart & Ioannis S. Minas
  - Research Associate: David Sterle
  - Student: Stephan Miller – PhD Research
Project 1 - Preventive Chemical Applications

Started March 20

- Topsin
- Vitaseal
- Vitaseal + Topsin
- 70% Latex
- 50% Latex + Topsin
- 70% Latex + Topsin

- JMS Oil + Lime Sulfur
- Nufilm + Lime Sulfur
- 70% Latex + Lime Sulfur
Project 2 - Preventive Chemical Applications

Vitaseal + Topsin
Latex + Topsin
Vitaseal + Lime Sulfur
Project 3 - Tolerance of cultivars to *Cytospora*

Tolerance under high pH and drought

- Glohaven (MI)
- Glowingstar (MI)
- Blushingstar (MI)
- Starfire (MI)
- Newhaven (MI)
- Flamin Fury PF19-007 (MI)
- Flamin Fury PF 23 (MI)
- Flamin Fury PF 24 (MI)
- Red Haven (MI)
- O'Henry (CA)
- Angelus (CA)
- Suncrest (CA)
**Project title:** Determining dispersal pathways of *Cytospora* for the development of management strategies for Cytospora canker on peaches

**PI:** Jane E. Stewart
– Student: Stephan Miller – PhD Research

Dispersal of spores/epidemiology
Wind, insects, pruning tools

Funding: USDA-CDA Specialty Crop
February 2019 – November 2020
Is inoculum spread similar in Colorado?

Pattern of disease if spread only by rain splash

Patterns we observe in orchards in Colorado

What are ways spores travel long distances? Wind, insects, humans?
The need for a molecular tool for *Cytospora* identification

- Spores are small and difficult to differentiate
- *Cytospora* is a slow grower and is outcompeted by other fungi

Spores of *Cytospora leucostoma* at 40X
Marker development as an epidemiology tool

- Genomes of several Colorado isolates were used to identify unique regions in *C. leucostoma*
- A digital drop molecular assay was developed and tested against closely-related species of *Cytospora*

Ibarra-Cabarello et al. Plant Disease. In prep
Molecular assay identifies only *C. leucostoma*

- Collect insects and test for vectors
- Test pruning shears
- Test nursery stock
Thanks!

Collaborators:
Greg Litus, Frank Stonaker, Harold Larsen, Jordge Lafantasie, Brady Shanahan, Anne Hess, Bruce Talbott, Cytospora working group – Larry Traubel and Steve Ela
Cytospora Working Group

Objectives

• Collaborate with local commercial growers to prioritize research efforts
• Prevention/Protection measures
• Disease management/spread measures
• Support in funding opportunities

Want to join? Contact:
Jane Stewart: Jane.Stewart@colostate.edu
Ioannis Minas: Ioannis.Minas@colostate.edu
Species differ in virulence

Biggs. 1986. Phytopathology
Species differ in location on tree

<table>
<thead>
<tr>
<th>Canker location</th>
<th>C. leucostoma</th>
<th>C. cincta</th>
<th>C. paraleucostoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branch</td>
<td>82.55</td>
<td>16.00*</td>
<td>1.15 ns</td>
</tr>
<tr>
<td>Trunk</td>
<td>94.50**</td>
<td>4.50</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Recent Survey of Colorado Orchards

• Estimate incidence and severity of *Cytospora* in major peach production areas of Western Colorado

• March/April 2015

• Conducted in Grand Valley, North Fork and Olathe areas
  – Focus on gathering data from a widespread area and range of orchard management
Methods

• Surveyed by orchard and variety
• Recorded presence/absence for every 10\textsuperscript{th} tree
• Counted and rated infection severity for every 50\textsuperscript{th} tree

• Interviewed growers
  – Irrigation
  – Orchard floor management
  – Pruning practices
  – Pre-plant practices (replant, fallow, rotation)
  – Frost protection
  – Fertilization practices
  – Pesticide application